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ARTIFICIAL INFECTION OF *AEDES* (*STEGOMYIA*) *AEGYPTI* (LINNAEUS) WITH FIVE AFRICAN ARBOVIRUSES

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Abstract: Experiments determined susceptibility of Ilobi strain of *Aedes* (*Stegomyia*) *aegypti* (Linnaeus) to infection by parental inoculation with 5 African arboviruses and determined inability to transmit to newborn mice by bite.

Experiments were carried out at the East African Virus Research Institute, Entebbe, Uganda, to determine the susceptibility of the Ilobi strain of *Aedes* (*Stegomyia*) *aegypti* (Linnaeus) to infection by parenteral inoculation with five African arboviruses and its ability to transmit to newborn mice by bite. Four of these viruses, Nyando, Tanga, *Eretmapodites* 124 and 147, were retained and multiplied in these mosquitoes. Lagos bat, the fifth virus, did not persist. Mosquito-to-mosquito transmission was attempted, and was successful with Nyando, Tanga and *Eretmapodites* 124 viruses. Attempts to transmit these four viruses to infant mice by bite were unsuccessful. Agar gel diffusion was very satisfactory for virus identification and much more rapid than neutralization or complement-fixation tests.

MATERIALS AND METHODS

Viruses

1. Nyando virus (MP 401), isolated from a pool of 110 *Anopheles funestus* (Williams et al. 1959): 13th mouse passage.
2. Tanga virus (MP 1329), isolated from a pool of 191 *A. funestus* (Woodall & Williams, in preparation): 1st mouse passage.
3. Lagos Bat virus (Boulger & Porterfield 1952), isolated from a pool of 6 brains of the fruit bat, *Eidolon helvum*: 13th mouse passage.
4. *Eretmapodites* 124, isolated from a pool of 205 *Eretmapodites* captured in Manera forest (Dr Série, Institute Pasteur d'Ethopie, 1963, personal communication): 25th mouse passage.
5. *Eretmapodites* 147, isolated from a pool of 131 *Eretmapodites* captured in the same forest (Dr Série Institute Pasteur d'Ethopie, 1963, personal communication): 19th mouse passage.

Viruses were diluted in sterile 0.75% bovine plasma albumin in phosphate buffered saline (pH 7.4) containing antibiotic (BAPS).

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Mice

Newborn Swiss albino mice, originally obtained from Carworth Farms, New York U.S.A. were used routinely for inoculation with mosquito pools. Virus titrations were made by intracerebral inoculation of infant mice with 0.1 ml of serial tenfold dilutions. Transmission attempts with newborn mice were made by exposing them to bites of infected mosquitoes on top of the cage, held firmly under a petri dish padded with cotton wool.

Mosquitoes

The Ilobi strain of *Aedes* (*Stegomyia*) *aegypti* (Linnaeus), maintained as a laboratory colony for some years at the East African Virus Research Institute, was used. Females were used three days after emergence from the pupae. After inoculation, mosquitoes were kept in a Barraud cage at constant temperature (29 °C) and humidity (70%), fed on raisins and provided with water. Mosquitoes, in lots of 60, were aspirated, anaesthetized with carbon dioxide, and injected parenterally (Merrill & Ten Broeck 1935, Hurlbut 1951, Varma 1960, Nye 1960) through the thorax with a very thin entomological needle (Corbet 1962).

Virus content estimation

For estimation of the virus content of mosquitoes, 10 were ground in 1 ml of BAPS, then spun at 2500 rpm for 10 minutes in a refrigerated centrifuge (International P.R.2, angle head 823a) and the supernates titrated intracerebrally in newborn mice and also injected into a new batch of 50 mosquitoes. End-points were calculated by the method of Reed & Muench (1938) and expressed as the reciprocal of the logarithm of the dilutions.

Virus identification

Virus identification in mouse brain material was carried out throughout each experiment using an agar gel precipitation test, based on the Clarke method (1962, 1964). Difco Noble agar in borate buffer pH 7.4, 5 ml of a 0.75% final concentration, was poured into 5 cm plastic petri dishes. Then a central and 6 surrounding wells, 5 mm in diameter and 3.5 mm apart, were punched. The central well was routinely filled with serum and the peripheral wells with the antigen to be tested. Sera and the source virus used in the test were pretested several times to ensure that they produced adequate

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lines. The infected mouse brain material, used as antigen, was a 20% suspension in BAPS. The reaction was allowed to proceed at a constant temperature of 29 °C.

Virus multiplication studies with A. aegypti

The 60 *A. aegypti*, inoculated with brain material on day 0 were called pool no. 1. Brain material was titrated in mice before mosquito inoculation. Immediately after inoculation, 10 mosquitoes were ground and titrated for virus content. On days, 5, 10, 15 and 20, 10 mosquitoes from pool no. 1 were ground up and centrifuged. The supernate was divided in two parts. Tenfold dilutions, up to 10^{-5} were made from the first part and injected intracerebrally into newborn mice. The second part was used in an attempt to produce multiplication in a new batch of mosquitoes.

Transmission attempts

On days 5, 10, 15, and 20, *A. aegypti* from pool no. 1 were allowed to feed on 10 newborn mice for 2 hours. These mice were examined carefully daily for alopecia or sickness and kept for 21 days.

Mosquito-to-mosquito transmission

On days 5, 10, 15, and 20, the second part of the supernate was inoculated, following the procedure described above, into 50 new female *A. aegypti*. These new mosquito pools were numbered 2, 3, 4, and 5. The injected mosquitoes were kept for 10 days. Then 10 of them were triturated, the supernatant being inoculated into newborn mice for virus content.

RESULTS

Nyando virus (*Strain MP* 401)

Titration of the preinoculation brain material gave a titre of $\geqq 6$ log LD_{50}/.01 ml. Ten *A. aegypti*, ground immediately after parenteral inoculation in 1 ml of BAPS, showed a titre of 1.8 log LD_{50}/.01 ml. Titres of virus present in pool no. 1 on days 5, 10, 15, and 20 after parenteral inoculation, were of 1.7, 3.1, 1.8, and 2.1. log LD_{50}/.01 ml. Those antigens gave positive results in gel diffusion when tested against a Nyando hyperimmune mouse antiserum. The result of mosquito-to-mosquito transmission attempts were negative with pools 2, 4, and 5, and were positive with pool 3 which gave a virus titre of 1.0 log LD_{50}/.01 ml.

None of the mice fed on by mosquitoes on days 5, 10, 15 and 20 showed any signs of illness. The sera from all mice groups, bled five weeks after exposure, did not contain any complement-fixing antibodies when tested against Nyando antigen.

Tanga virus (*Strain MP* 1329)

Inoculum titration in 2-day-old mice gave a titre of 5.2 log LD_{50}/.01 ml, 10 *A. aegypti* from pool no. 1 (MP 5163) ground immediately after parenteral inoculation in 1 ml of BAPS showed a titre of 1.3 log LD_{50} .01 ml. Virus multiplication in *A. aegypti* was at its highest 10 days after artificial infection, giving a titre of 3.0 log LD_{50}/.01 ml. Tanga virus, identified by the gel diffusion technique, was also recovered from pool no. 1 on days 5, 15, and 20, showing titres around 2.4 log.

Mosquito-to-mosquito transmission attempts were positive, and virus titre was about 2.0 log in each pool 2, 3, 4, and 5. None of the mice fed on by mosquitoes on days 5, 10, 15, and 20 showed any signs of illness except one. The brain of this mouse, belonging to the day 5 group, was kept and identification carried out in gel diffusion. The results were negative. A passage was immediately made but no virus was recovered. No antibody studies were made, mouse groups having been discarded in error.

In the course of a similar experiment using Tanga virus, an assay was made to detect virus directly in mosquitoes, using the gel diffusion technique. In this case 20 *A. aegypti* were ground in .6 ml of diluent and, without centrifugation, put in the antigen wells against the same hyperimmune Tanga serum. Normal uninfected mosquito material was used as a control. Positive results were obtained with mosquitoes known to contain virus.

Lagos Bat virus

Brain material used for mosquito inoculation had a titre of 4.7 log LD_{50}/.01 ml in newborn mice; 10 *A. aegypti* ground immediately after parenteral infection showed a titre of 2.1 log LD_{50}. Multiplication studies in mosquitoes gave negative results. We could not detect Lagos bat virus on days 5, 10, 15 or 20. For transmission attempts, anaesthetized (Sagatal .08 ml per bat) adult fruit bats (*E. helvum*) were fed on by mosquitoes on days 5, 10, and 15. Blood samples were taken from the bats by intracardiac puncture on days 2, 3, and 4 and then inoculated intracerebrally into newborn mice. All results were negative. Lagos bat virus showed very small and poorly contrasted lines in gel diffusion. The serum used (guinea pig hyperimmune) probably contained very little precipitating antibody.

Eretmapodites 124

Titration of brain inoculum into 2-day-old mice gave a titre of $\geqq 7$ log LD_{50}/.01 ml, 10 *A. aegypti* ground immediately after parenteral inoculation showed a titre of 1.5 log LD_{50}/.01 ml. Multiplication of *Eretmapodites* 124 virus in *A. aegypti*, inocu-

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lated on day 0 with infected mouse brain material, was at its highest 10 days after artificial infection, with titre reaching 5 log. At 5, 15, and 20 days after artificial infection, virus content estimation gave titres of around 3.0 log. The mosquito-to-mosquito transmission attempts were positive in pools 3 and 4 only. Pools 2 and 5 were negative. Identification of *Eretmapodites* 124 virus was by the agar gel diffusion technique. None of the mice fed on by mosquitoes on days 5, 10, 15 and 20 showed any signs of illness. Thesera from all mouse groups, bled 28 days after exposure, did not contain any complement-fixing antibodies when tested against *Eretmapodites* 124 antigen.

Eretmapodites 174

Titration of the brain material in 2-day-old mice showed a titre of $\geqq$ 7 log LD_{50}/.01 ml, 10 *A. aegypti* ground immediately after parenteral inoculation in 1 ml of BAPS showed a titre of 3.0. But in spite of this rather rich inoculum, virus multiplication in *A. aegypti* was comparatively low, showing a maximum titre of 2.3 log LD_{50}/.01 ml, 10 days and 15 days after artificial infection. Mosquito-to-mosquito transmission attempts were negative. None of the mice fed on by mosquitoes on days 5, 10, 15, and 20 showed any signs of illness. The sera from all mouse groups, bled 28 days after exposure, did not contain any complement-fixing antibodies when tested against *Eretmapodites* 147 antigen.

DISCUSSION

Of the five viruses studied, four have been shown to multiply within the mosquitoes. These viruses were Nyando, Tanga, *Eretmapodites* 124, and 147. This multiplication provides secondary proof that these viruses belong to the arthropod-borne group of viruses. It also shows that multiplication within the mosquito is sufficient to compensate for the fall in virus titre in mosquito-to-mosquito transmission. In each case the quantity of infective virus particles injected, expressed as the inverse of the log LD_{50} /.01 ml in mice, was important.

Thus for Nyando, reinfection of a new lot of mosquitoes occurs when the titre of the virus in the first lot is 3.1 log LD_{50}/.01 ml, but a titre of 2.1 logs is insufficient. The same applies for *Eretmapodites* 124. Lagos bat virus, however, was not recovered from mosquitoes on post-inoculation day 5. One assumes that no multiplication of this virus occurred in *A. aegypti*. This also was found by Boulger & Porterfield (1958).

In spite of an adequate virus titre in mosquitoes, no transmission occurred from mosquito to mouse when mosquitoes were allowed to feed on newborn mice. *A. aegypti* does not appear to be an efficient vector of these viruses, perhaps because of a salivary barrier impeding salivary gland infection (Chamberlain & Sudia 1961). The use of gel diffusion as a means of virus identification is very satisfactory and is much more rapid than neutralization or complement-fixation tests.

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